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Award Number: DAMD17-99-1-9297

TITLE: Soy, Probiotics, and Breast Cancer Prevention

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REPORT DATE: October 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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20020717 039

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 2001	3. REPORT TYPE AND DATES COVERED Annual (15 Sep 00 - 14 Sep 01)	
4. TITLE AND SUBTITLE Soy, Probiotics, and Breast Cancer Prevettention			5. FUNDING NUMBERS DAMD17-99-1-9297	
6. AUTHOR(S) Mindy S. Kurzer, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Minnesota Minneapolis, Minnesota 55415-1226 E-Mail: mkurzer@umn.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) The purpose of this study is to investigate the effects of soy and probiotic consumption on estrogen and phytoestrogen metabolism. The methods include <i>in vitro</i> studies to determine the intestinal microflora responsible for phytoestrogen metablism, and a human feeding study in which 20 postmenopausal breast cancer survivors and 20 controls consume four different dietary supplements for six weeks each. The supplements were: 1) soy powder; 2) soy powder + probiotic; 3) milk power; 4) milk powder plus probiotic. Urine was collected for three days before the study began and for three days at the end of each diet period, for evaluation of urinary estrogen and phytoestrogen metabolites. Food records were collected on the same days as the urines. Feces were collected before the study began and at the end of each diet period, for evaluation of intestinal microflora profiles. At this point, both <i>in vitro</i> and human studies have been completed. All subjects have completed the human feeding study, urines have been collected and processed and fecal samples have been analyzed. During the final year, the urine samples will be analyzed, data analyses will be performed, and manuscripts will be prepared for publication.				
14. SUBJECT TERMS Breast Cancer, probiotic, phytoestrogen, soy				15. NUMBER OF PAGES 6
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified		20. LIMITATION OF ABSTRACT Unlimited

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INTRODUCTION

Although most research on dietary prevention of breast cancer in Asia focuses on soy consumption, the Asian diet also contains large quantities of bacteria (probiotics) in fermented foods. For this project, we hypothesize that consumption of probiotic with soy may enhance soy's cancer-preventive effects by shifting phytoestrogen metabolism in a beneficial direction. The purpose of this study is to investigate the effects of soy and probiotic consumption on estrogen and phytoestrogen metabolism. The specific aims are to identify the intestinal bacteria responsible for phytoestrogen metabolism, via *in vitro* studies, and to determine the independent and interactive effects of soy and probiotic consumption on estrogen and phytoestrogen metabolism in women who have had breast cancer and controls. First, *in vitro* studies were performed to determine which intestinal microflora are responsible for production of the phytoestrogen metabolite equol, which has been associated with low risk of breast cancer. Next, a randomized crossover human study was performed, in which 20 postmenopausal breast cancer survivors and 20 age-matched controls consumed 4 different dietary supplements for six weeks each, separated by 2-week washout periods. The supplements were: 1) soy powder; 2) soy powder + probiotic; 3) milk powder; 4) milk powder plus probiotic. Urine was collected for 3 days before the study began, and for 3 days at the end of each diet period, for evaluation of urinary estrogen and phytoestrogen metabolites. To assure that energy and nutrient intakes did not differ between diet periods, food records were collected on the same days as the urines. Feces were collected once before the study began, and once at the end of each washout and diet period, for evaluation of intestinal microflora profiles.

BODY

According to the original statement of work, the following tasks were to be performed during the second year of this project:

1. Determine the independent and interactive effects of soy and probiotic consumption on intestinal microflora, urinary phytoestrogens and estrogen metabolites in women who have had breast cancer and controls.
 - Perform randomized crossover feeding study with women who have never had breast cancer (control subjects), perform fecal assays, process and store urines
 - Analyze urines from control subjects for urinary phytoestrogens and estrogen metabolites, using GC/MS methodology
 - Recruit 18 breast cancer survivors
 - Perform randomized crossover feeding study with breast cancer survivors, perform fecal assays, process and store urines

We have made the following changes in the organization and scope of the study as originally written:

- Instead of studying the control women in the first year and the breast cancer survivors in the second and third years, we decided that it was better from a statistical and practical viewpoint to begin recruiting both groups of women during the first year. The advantages of this change are 1) comparison between the two groups is not affected by different recruiting years; and 2) we had more time to recruit breast cancer survivors, which we knew would be difficult due to our strict exclusionary criteria.
- We modified the design of the study to add a control milk powder. The original design had three diet periods: soy powder alone, soy + probiotic, and probiotic alone. The modified design has four diet periods: soy powder alone, soy + probiotic, milk powder alone, and milk powder + probiotic. We believe that this has added considerable strength to the original design. We also added a placebo capsule to be taken during the soy alone and milk powder alone diet periods. Unfortunately, there was a problem with the placebo capsules, which were found to be contaminated with probiotic. We discontinued their use as soon as this was discovered, and diet periods during which placebo capsules had been taken were repeated. (*This change was noted in the year 1 annual report*)
- Our statistician advised that 20 controls and 20 breast cancer survivors would be easier to randomize in a balanced design for this study. Therefore, instead of 18 subjects per group, we recruited and completed 20 subjects per group.

We have successfully stayed on schedule during the second year. Eighty-five women were interviewed, of which 61 women underwent health screen evaluations for the study. Of those women, 53 were enrolled in the study. Fifteen women dropped out of the study as a result of difficulties due to dietary restrictions (1), peri-menopausal status (1), unrelated medical issues (4), incompatible travel plans (4), and time conflicts (5). Forty subjects completed the study (20 controls and 20 breast cancer survivors), although three breast cancer survivors did not provide complete data: two subjects completed 3 diet periods and one subject completed 2 diet periods. All other subjects completed all four dietary periods. Fecal samples have been analyzed for microflora profiles, and data have been entered into an Excel spreadsheet. Data analysis is underway. All urine samples have been collected, processed and stored as stated in the original proposal.

KEY RESEARCH ACCOMPLISHMENTS

- Successfully recruited the proposed number of subjects and completed the human feeding study
- Completed collection of all urine samples, processed and stored urine
- Collected and analyzed all fecal samples

REPORTABLE OUTCOMES

None at this time

CONCLUSIONS

The human feeding study has been successfully carried out and all subjects have completed the study. All biological samples have been analyzed or stored as stated in the original study design. During the third year, we will complete the urinary phytoestrogen and estrogen metabolite analyses, perform data analyses and prepare manuscripts for publication. We will have no problem completing all tasks by the end of the grant period. At this point there are no reportable data from which to draw conclusions.

REFERENCES

None

APPENDICES

None